

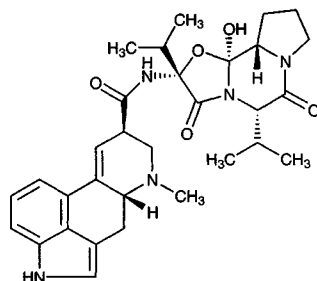
Ergocornine

Molecular formula: $C_{31}H_{39}N_5O_5$

Molecular weight: 561.68

CAS Registry No.: 564-36-3

Merck Index: 3685



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cycizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipranone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flvoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscipine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxibenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

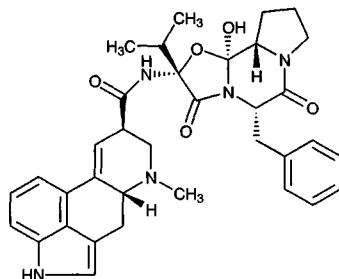
Ergocristine

Molecular formula: $C_{35}H_{39}N_5O_5$

Molecular weight: 609.73

CAS Registry No.: 511-08-0

Merck Index: 3687



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, buprenorphine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pi-

renzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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Ergocristinine

Molecular formula: $C_{35}H_{39}N_5O_5$

Molecular weight: 609.73

CAS Registry No.: 511-07-9

Merck Index: 3688

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortrip-

tyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranylecypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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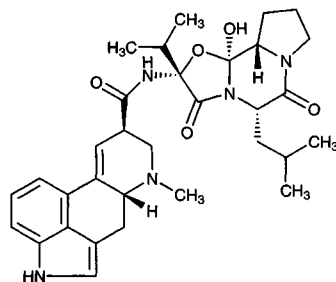
Ergocryptine

Molecular formula: $C_{32}H_{41}N_5O_5$

Molecular weight: 575.71

CAS Registry No.: 511-09-1 (α) 20315-46-2 (β)

Merck Index: 3689



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 $\mu\text{g/mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipamnone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine,

droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampramide, phenazocine, phenbutrazate, phenidimetazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindone, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleannamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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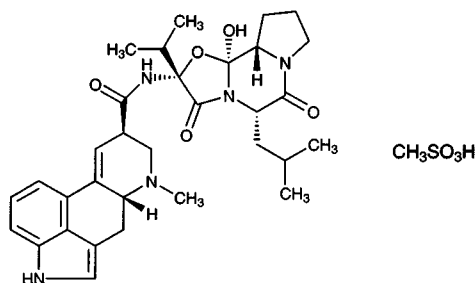
Ergoloid mesylates

Molecular formula: $C_{36}H_{45}N_5O_6S$

Molecular weight: 707.85

CAS Registry No.: 8067-24-1

Merck Index: 3692



Dihydroergocornine $R = CH(CH_3)_2$

Dihydroergocristine $R = CH_2C_6H_5$

Dihydro- α -ergocryptine $R = CH_2CH(CH_3)_2$

Dihydro- β -ergocryptine $R = CH(CH_3)CH_2CH_3$

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μ L 1 μ g/mL dihydroergotamine mesylate in water + 30 μ L 5 M NaOH + 7 mL chloroform, shake on a reciprocal shaker for 10 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L mobile phase, inject a 10–30 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 10 μ m RP-8 (Merck)

Mobile phase: MeCN:buffer 60:40 (Buffer was 9 mM NaH_2PO_4 and 9 mM Na_2HPO_4 , pH 7.2.)

Flow rate: 1

Injection volume: 10-30

Detector: UV 223 or F ex 295 em 350

CHROMATOGRAM

Retention time: 7.9 (dihydroergocristine)

Internal standard: dihydroergotamine mesylate (5.2)

Limit of detection: 0.5-0.7 ng/mL (F), 5-10 ng/mL (UV)

OTHER SUBSTANCES

Simultaneous: dihydroergocornine, dihydroergocryptine

KEY WORDS

plasma; rat

REFERENCE

Zecca,L.; Bonini,L.; Bareggi,S.R. Determination of dihydroergocristine and dihydroergotamine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1983**, 272, 401-405.

SAMPLE

Matrix: blood, urine, tissue

Sample preparation: Homogenize tissue with 4 volumes of water, centrifuge. 1 mL Plasma, urine, or tissue homogenization supernatant + 50 μ L 1 (plasma) or 10 (urine, tissue) μ g/mL α -dihydroergocristine + 100 μ L 1 M HCl, vortex, add 5 mL hexane, extract, centrifuge. Remove the aqueous phase and add it to 100 μ L 2 M NaOH, extract with 7 mL chloroform, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 70 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4 5 μ m LiChrospher 100 RP 18

Mobile phase: MeCN:buffer 43:57 (Buffer was 10 mM pH 7.2 Na₂HPO₄/KH₂PO₄.)

Flow rate: 1

Injection volume: 50

Detector: F ex 295 em 350

CHROMATOGRAM

Retention time: 8.9 (dihydroergocriptine)

Internal standard: α -dihydroergocristine (10.2)

Limit of detection: 2 ng/g (tissue), 10 ng/mL (urine), 0.1 ng/mL (plasma)

KEY WORDS

rat; plasma; kidney; heart; lung; spleen; liver; brain; pharmacokinetics

REFERENCE

Coppi,G.; Silingardi,S. Pharmacokinetics of α -dihydroergocriptine in rats after single intravenous and single and repeated oral administrations, *Biopharm.Drug Dispos.*, **1995**, 16, 333-342.

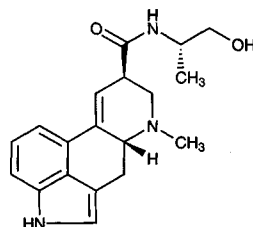
Ergonovine

Molecular formula: C₁₉H₂₃N₃O₂

Molecular weight: 325.41

CAS Registry No.: 60-79-7, 129-51-1 (maleate)

Merck Index: 3694



SAMPLE

Matrix: blood

Sample preparation: 300 μ L Plasma + 300 μ L MeCN, centrifuge at 11000 g for 4 min, inject a 100 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: 75 \times 2.1 10 μ m pellicular reversed phase (Chrompack no. 028653)

Column: 250 \times 4.6 5 μ m Spherisorb 5-ODS

Mobile phase: MeCN:buffer 35:65 (Buffer was 67 mM KH_2PO_4 containing 0.5 mL/L (?) triethylamine.)

Flow rate: 1.2

Injection volume: 100

Detector: F ex 315 em 430

CHROMATOGRAM

Retention time: 5.26

Limit of quantitation: 75 pg/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

de Groot, A.N.J.A.; Vree, T.B.; Hekster, Y.A.; Baars, A.M.; Van den Biggelaar-Martea, M.; van Dongen, P.W.J. High-performance liquid chromatography of ergometrine and preliminary pharmacokinetics in plasma of men, *J.Chromatogr.*, **1993**, 613, 158–161.

SAMPLE

Matrix: formulations

Sample preparation: Dilute formulation 1:10. Remove a 1 mL aliquot and add it to 1.5 mL water and 2.5 mL 20 μ g/mL ephedrine, inject an 80 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeCN:buffer 35:65 containing 0.05% sodium tetradecyl sulfate (Buffer was 0.83 mM phosphoric acid adjusted to pH 5.0 with triethylamine. Use a 50 \times 4.6 5-25 μ m LiChroprep Si 60 column before the injector. Wash column with MeCN:83 mM phosphoric acid 40:60 after use.)

Flow rate: 2.5

Injection volume: 80

Detector: UV

CHROMATOGRAM

Retention time: 18

Internal standard: ephedrine (10)

OTHER SUBSTANCES

Simultaneous: oxytocin

KEY WORDS

injections

REFERENCE

Pask-Hughes, R.A.; Corran, P.H.; Calam, D.H. Assay of the combined formulation of ergometrine and oxytocin by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, 214, 307–315.

SAMPLE

Matrix: formulations

Sample preparation: Injections. 1 mL Injection (200 μ g/mL) + 300 mg NaCl + 200 μ L 10% ammonia + 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 20 μ g/mL 17 α -hydroxyprogesterone in MeOH, inject a 20 μ L aliquot. Tablets. Weigh out amount of powdered tablets equivalent to about 200 μ g compound, add 1 mL water, sonicate for 2 min, add 300 mg

NaCl, add 200 μ L 10% ammonia, add 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 20 μ g/mL 17 α -hydroxyprogesterone in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.5 μ m LiChrosorb RP-18

Mobile phase: MeCN:50 mM pH 3.5 acetate buffer 40:60 containing 1.5 mM triethylamine

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

Internal standard: 17 α -hydroxyprogesterone (12)

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, methylergonovine

Noninterfering: ascorbic acid

KEY WORDS

injections; tablets

REFERENCE

Tokunaga,H.; Kimura,T.; Kawamura,J. Determination of ergometrine maleate and methylergometrine maleate in pharmaceutical preparations by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1983**, *31*, 3988–3993.

SAMPLE

Matrix: formulations

Sample preparation: Grind tablet, add 100 μ L 90% formic acid for each 100 μ g ergotamine tartrate, swirl to thoroughly wet sample, make up to 100 mL with MeOH, mix, filter (paper), dilute filtrate with MeOH (if necessary) so that the ergotamine tartrate concentration is 3 mg/L, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 C18 (Alltech)

Mobile phase: MeCN:water:triethylamine 70:30:0.05

Flow rate: 1

Injection volume: 20

Detector: F ex 250 em 430

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES

Simultaneous: ergotamine, ergotaminine

Noninterfering: caffeine

KEY WORDS

tablets

REFERENCE

Cieri,U.R. Determination of ergotamine tartrate in tablets by liquid chromatography with fluorescence detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 538–540.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES**Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 1.2**OTHER SUBSTANCES**

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipamone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, mornazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenamproide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piriramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE**Matrix:** solutions**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.**HPLC VARIABLES****Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78**Flow rate:** 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.69

REFERENCE

Roos, R.W.; Lau-Cam, C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, 370, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 25–150 µL aliquot.

HPLC VARIABLES

Column: 250 mm long 5 µm Hypersil C18 ODS

Mobile phase: MeCN:10 mM ammonium carbonate 30:70

Flow rate: 2

Injection volume: 25–150

Detector: F ex 328 em 415

CHROMATOGRAM

Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: methylergonovine (methylegometrine), methysergide

REFERENCE

Bredberg, U.; Paalzow, L. Pharmacokinetics of methysergide and its metabolite methylegometrine in the rat, *Drug Metab. Dispos.*, **1990**, 18, 338–343.

SAMPLE

Matrix: wheat

Sample preparation: Grind wheat to pass 2 mm screen. 25 g Ground wheat + 10 mL 4% ammonia in water + 100 mL ethyl acetate, shake vigorously on a wrist-action shaker for 15 min, filter (paper). Extract 50 mL filtrate twice with 25 mL 1% sulfuric acid. Combine aqueous extracts, add 50 mL 4% ammonia in water, extract twice with 25 mL dichloromethane shaking gently for 30 s each time. Dry extracts over 10–20 g anhydrous sodium sulfate for 10 min in the dark, filter, wash solid with 25 mL dichloromethane. Evaporate filtrate to near dryness at 40° under reduced pressure, rinse into a tube with two 2 mL portions of dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 10 µm PRP-1 polystyrene-divinylbenzene (Hamilton)

Mobile phase: MeCN:water 45:55 containing 6.6 g/L (NH₄)₂HPO₄

Flow rate: 0.3

Injection volume: 20

Detector: F ex not specified em 418 (cut-off filter)

CHROMATOGRAM

Retention time: 7

Limit of detection: < 18 ng/g

OTHER SUBSTANCES

Extracted: ergonovine, ergotamine, ergocryptine, ergocristine, ergotamine, ergocryptine, ergocristine

REFERENCE

Ware, G.M.; Carman, A.S.; Francis, O.J.; Kuan, S.S. Liquid chromatographic determination of ergot alkaloids in wheat, *J. Assoc. Off. Anal. Chem.*, **1986**, 69, 697–699.

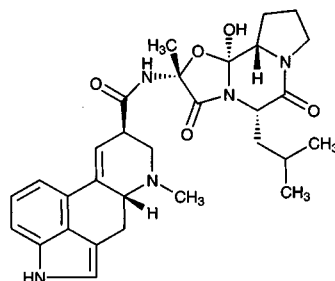
Ergosine

Molecular formula: C₃₀H₃₇N₅O₅

Molecular weight: 547.65

CAS Registry No.: 561-94-4

Merck Index: 3695



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenamproide, phenazocine, phenbutrazate, phenidimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylidamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, tra-

zodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

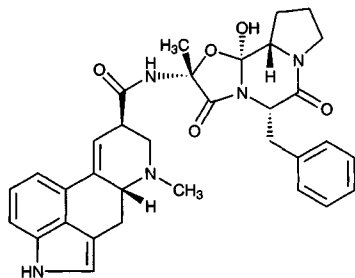
Ergotamine

Molecular formula: $C_{33}H_{35}N_5O_5$

Molecular weight: 581.67

CAS Registry No.: 113-15-5, 379-79-3 (tartrate)

Merck Index: 3703



SAMPLE

Matrix: formulations

Sample preparation: Grind tablet, add 100 μ L 90% formic acid for each 100 μ g ergotamine tartrate, swirl to thoroughly wet sample, make up to 100 mL with MeOH, mix, filter (paper), dilute filtrate with MeOH (if necessary) so that the ergotamine tartrate concentration is 3 mg/L, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 C18 (Alltech)

Mobile phase: MeCN:water:triethylamine 70:30:0.05

Flow rate: 1

Injection volume: 20

Detector: F ex 250 em 430

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: ergonovine, ergotamine

Noninterfering: caffeine

KEY WORDS

tablets

REFERENCE

Cieri, U. R. Determination of ergotamine tartrate in tablets by liquid chromatography with fluorescence detection, *J. Assoc. Off. Anal. Chem.*, **1987**, 70, 538–540.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotinine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclizine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE**Matrix:** solutions**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.**HPLC VARIABLES****Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38**Flow rate:** 1.5**Injection volume:** 10**Detector:** UV**CHROMATOGRAM**

Retention time: k' 1.55

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 µm Nucleosil C18

Mobile phase: MeCN:10 mM ammonium carbonate 50:50

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: impurities, ergocristine, ergosine, 8-hydroxyergotamine

REFERENCE

Gazdag,M.; Szepesi,G. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. IV. Selection of most applicable separation system, *J.Chromatogr.*, **1989**, 464, 279–288.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak phenyl

Mobile phase: MeCN:0.1% pH 3.25 acetic acid containing 1.25 mM heptanesulfonic acid 65:35

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

REFERENCE

Fernández Otero,G.C.; Lucangioli,S.E.; Carducci,C.N. Adsorption of drugs in high-performance liquid chromatography injector loops, *J.Chromatogr.A*, **1993**, 654, 87–91.

SAMPLE

Matrix: solutions

Sample preparation: Pass 20 mL of a solution in water (?) through an Empore C18 SPE disc. Wash with 2.5 mL water, add 1 mL MeCN:25 mM pH 3.0 phosphate buffer 65:35, let soak for 3 min, elute, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb S5 ODS2

Mobile phase: MeCN:10 mM KH₂PO₄ 30:70

Flow rate: 1.3

Detector: UV 254

OTHER SUBSTANCES

Noninterfering: excipients

KEY WORDS

comparison with capillary electrophoresis; SPE

REFERENCE

Lucangioli,S.E.; Rodriguez,V.G.; Fernandez Otero,G.C.; Vizioli,N.M.; Carducci,C.N. Development and validation of capillary electrophoresis methods for pharmaceutical dissolution assays, *J.Capillary Electrophor.*, **1997**, 4, 27–31.

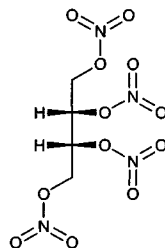
SAMPLE**Matrix:** wheat

Sample preparation: Grind wheat to pass 2 mm screen. 25 g Ground wheat + 10 mL 4% ammonia in water + 100 mL ethyl acetate, shake vigorously on a wrist-action shaker for 15 min, filter (paper). Extract 50 mL filtrate twice with 25 mL 1% sulfuric acid. Combine aqueous extracts, add 50 mL 4% ammonia in water, extract twice with 25 mL dichloromethane shaking gently for 30 s each time. Dry extracts over 10-20 g anhydrous sodium sulfate for 10 min in the dark, filter, wash solid with 25 mL dichloromethane. Evaporate filtrate to near dryness at 40° under reduced pressure, rinse into a tube with two 2 mL portions of dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES**Column:** 150 × 4.6 10 µm PRP-1 polystyrene-divinylbenzene (Hamilton)**Mobile phase:** MeCN:water 45:55 containing 6.6 g/L (NH₄)₂HPO₄**Flow rate:** 0.3**Injection volume:** 20**Detector:** F ex not specified em 418 (cut-off filter)**CHROMATOGRAM****Retention time:** 15**Limit of detection:** < 95 ng/g**OTHER SUBSTANCES****Extracted:** ergonovinine, ergonovine, ergocryptine, ergocristine, ergotaminine, ergocryptinine, ergocristinine**REFERENCE**

Ware, G.M.; Carman, A.S.; Francis, O.J.; Kuan, S.S. Liquid chromatographic determination of ergot alkaloids in wheat, *J. Assoc. Off. Anal. Chem.*, **1986**, 69, 697-699.

Erythrityl tetranitrate

Molecular formula: C₄H₆N₄O₁₂**Molecular weight:** 302.11**CAS Registry No.:** 7297-25-8**Merck Index:** 3716**SAMPLE****Matrix:** formulations

Sample preparation: Powder tablets, weigh out a portion equivalent to 3 mg erythrityl tetranitrate, add to 10 mL 75 µg/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

HPLC VARIABLES**Guard column:** 40 × 4.6 µBondapak C18/Corasil**Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:water 40:60**Flow rate:** 1**Injection volume:** 20**Detector:** UV 220**CHROMATOGRAM****Retention time:** 31**Internal standard:** nitroglycerin (14)

OTHER SUBSTANCES

Simultaneous: pentaerythritol tetranitrate, isosorbide dinitrate

KEY WORDS

tablets

REFERENCE

Olsen, C.S.; Scroggins, H.S. High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms, *J.Pharm.Sci.*, **1984**, 73, 1303-1304.

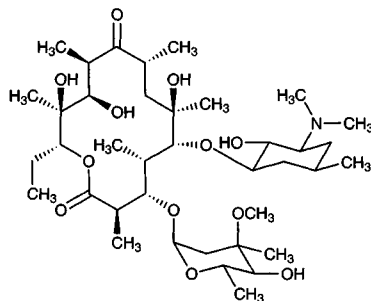
Erythromycin

Molecular formula: $C_{37}H_{67}NO_{13}$

Molecular weight: 733.94

CAS Registry No.: 114-07-8, 41342-53-4 (ethylsuccinate), 96128-89-1 (acistrate), 3521-62-8 (estolate), 304-63-2 (gluheptonate), 23067-13-2 (gluheptonate), 3847-29-8 (lactobionate), 134-36-1 (propionate), 643-22-1 (stearate), 84252-03-9 (stinoprate)

Merck Index: 3720

**SAMPLE**

Matrix: blood, tissue

Sample preparation: Homogenize (Physoctron) liver with 4 volumes of ice-cold saline. Evaporate 100 μ L 3 μ g/mL oleandomycin in MeOH to dryness under dry nitrogen. Put 200 μ L plasma or liver homogenate into the tube. Add 2 mL MTBE and 5 μ L 1 M NaOH and shake mechanically for 5 min. Centrifuge at 1500 g for 10 min, transfer the upper layer transfer into a glass tube and evaporate it to dryness under dry nitrogen. Rinse the inner wall of the tube with 200 μ L MeOH and evaporate to dryness. Dissolve the residue in 30 μ L MeOH and inject a 10 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Cosmosil 5-C18 (Nacalai Tesque)

Mobile phase: MeCN:100 mM pH 6.6 sodium acetate buffer 50:50

Flow rate: 0.6

Injection volume: 10

Detector: E, 1.1 V, Ag/AgCl reference electrode IS oleandomycin (8.5)

CHROMATOGRAM

Retention time: 12.8

Limit of detection: 100 μ g/mL (plasma); 0.5 μ g/g (liver)

KEY WORDS

rat; plasma; liver; pharmacokinetics

REFERENCE

Hanada, E.; Ohtani, H.; Kotaki, H.; Sawada, Y.; Iga, T. Determination of erythromycin concentrations in rat plasma and liver by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1997**, 692, 478-482.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Erythromycin estolate bulk. Dissolve the bulk powder in mobile phase B for a final concentration of 5-6 mg/mL and sonicate for 30 s. Inject an aliquot. Erythromycin estolate capsules. Powder the contents of capsules. Prepare a 5 mg/mL suspension in mobile

phase B, sonicate for 2 min, filter (0.45 μm), discard the first 5 mL of the filtrate. Inject an aliquot. Erythromycin ethylsuccinate powder. Prepare as described for estolate. Erythromycin ethylsuccinate tablets. Powder the tablets, prepare a 15 mg/mL suspension in mobile phase B, sonicate for 2 min, filter (0.45 μm). Discard the first few mL of filtrate. Inject an aliquot. Erythromycin ethylsuccinate powder for oral suspension. Prepare a 500 mg/mL suspension in 100 mL mobile phase B, sonicate for 15 min, make up the supernatant to 100 mL with mobile phase B, filter (0.45 μm). Inject an aliquot. Erythromycin stearate powder. Prepare a 5-6 mg/mL solution in MeOH, inject an aliquot. Erythromycin stearate tablets. Powder the tablets and prepare a 30 mg/mL suspension in MeOH, sonicate for 5 min, filter (0.45 μm), discard the first few mL of the filtrate, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm Inertsil ODS-2

Column: 150 \times 4.6 5 μm Inertsil ODS-2 (A) or 250 \times 4.6 5 μm Inertsil ODS-2 (B)

Mobile phase: Gradient. A was MeCN:buffer 10:90. B was MeCN:buffer 75:25. A:B from 90:10 to 0:100 over 10 min, maintain at 0:100. (Prepare mobile phase A as follows. Mix 60 mL 200 mM pH 6.5 ammonium phosphate buffer with 60 mL 200 mM pH 6.5 tetrabutylammonium sulfate buffer and 200 mL water. Add 100 mL MeCN and make up to 1 L with water. Prepare mobile phase B as described for A except use 750 mL MeCN.)

Column temperature: 50

Flow rate: 1.3

Injection volume: 50

Detector: UV 205

CHROMATOGRAM

Retention time: 9.5 (estolate, column A), 14.5 (ethylsuccinate, column A), ca. 26 (stearate, column B), ca. 26 (glucopate, column B), ca. 26 (lactobionate, column B)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

powder; capsules; tablets

REFERENCE

Nasr, M.M.; Stanley, C.M High performance liquid chromatographic assay of erythromycin salts and esters in bulk and pharmaceutical dosage forms, *J. Liq. Chromatogr. Rel. Technol.*, **1998**, *21*, 1147-1160.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 \times 4 5 μm C18

Column: 125 \times 4 5 μm Lichrospher RP18

Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min

Flow rate: 0.5

Injection volume: 200

Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 442.3-619.4 in NCI and 734.4-576.2 in PCI

CHROMATOGRAM

Retention time: 6.4

Limit of detection: 50 ng/g

OTHER SUBSTANCES

Extracted: josamycin, spiramycin, tilmicosin, tylosin

KEY WORDS

muscle; cow; SPE

REFERENCE

Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *JAOAC Int.*, **1996**, 79, 397-404.

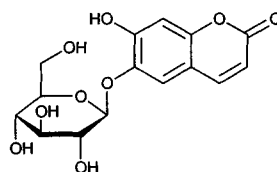
Esculin

Molecular formula: C₁₅H₁₆O₉

Molecular weight: 340.29

CAS Registry No.: 531-75-9

Merck Index: 3739



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM

Retention time: 5.277

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

Esmolol

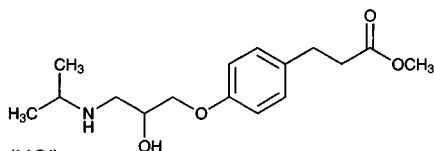
Molecular formula: $C_{16}H_{25}NO_4$

Molecular weight: 295.38

CAS Registry No.: 81147-92-4, 103598-03-4, 81161-17-3 (HCl)

Merck Index: 3741

Lednicer No.: 4 27



SAMPLE

Matrix: blood

Sample preparation: 1 mL Blood + 6 mL dichloromethane + 100 μ L 200 mM NaOH + 5 μ L 50 μ g/mL IS in water, vortex for 10 s, shake on a mechanical shaker for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 100 μ L 2.5 mM sulfuric acid, vortex for 1 min, centrifuge for 5 min, inject a 50 μ L portion of the aqueous layer.

HPLC VARIABLES

Guard column: 30 \times 3.2 37-53 μ m Whatman C18 guard column

Column: 100 \times 5 10 μ m Radial-Pak CN (Waters)

Mobile phase: MeOH:60 mM KH_2PO_4 :triethylamine 25:75:0.1 adjusted to pH 3.15 with 85% phosphoric acid

Flow rate: 1.8

Injection volume: 50

Detector: UV 221

CHROMATOGRAM

Retention time: 2.6

Internal standard: 3-methoxy-O-demethylencaïnide (3.9)

Limit of detection: 5 ng/mL

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amiodarone, atropine, caffeine, cimetidine, digoxin, diazepam, disopyramide (norpace), encainide, flecainide, imipramine, lidocaine, nimodipine, prazepam, procainamide, propafenone, propranolol, quinidine, theophylline

Interfering: captopril

KEY WORDS

pig; whole blood

REFERENCE

Fan,C.-D.; Zhao,H.; Chow,M.S.S. Simple and rapid high-performance liquid chromatographic assay for esmolol, *J.Chromatogr.*, **1991**, 570, 217-223.

SAMPLE

Matrix: blood

Sample preparation: 1 mL plasma + 5 mL dichloromethane, shake for 10 min, centrifuge at 4° at 1900 g for 10 min. Remove 4 mL of the organic phase and add it to 600 μ L 100 mM pH 2.8 NaH_2PO_4 , shake, centrifuge, inject a 100 μ L aliquot. (To determine metabolites mix 500 μ L aqueous phase (left after initial extraction) with 500 μ L 7% perchloric acid, centrifuge for 5 min, inject a 100 μ L aliquot of the supernatant.)

HPLC VARIABLES

Guard column: 5 μ m Lichrocart 4-4 RP18 (Merck)

Column: 150 \times 3.9 5 μ m Nova-Pak C18

Mobile phase: MeCN:10 mM pH 2.4 NaH_2PO_4 40:60 containing 0.2 mM sodium dodecylsulfate (For metabolites use MeCN:10 mM pH 2.4 NaH_2PO_4 17.5:82.5 containing 0.2 mM sodium dodecylsulfate.)

Flow rate: 1

Injection volume: 100

Detector: UV 229

CHROMATOGRAM

Retention time: 5.4

Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma

REFERENCE

Jahn,P.; Eckrich,B.; Schneidrowski,B.; Volz-Zang,C.; Schulte,B.; Mutschler,E.; Palm,D. β_1 -Adrenoceptor subtype selective antagonism of esmolol and its major metabolite in vitro and in man. Investigations using trisacrylphosphate as red blood cell carboxylesterase inhibitor, *Arzneimittelforschung*, **1995**, 45, 536–541.

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water to an expected esmolol hydrochloride concentration of 10 $\mu\text{g/mL}$. Remove a 100 μL aliquot and add it to 100 μL 15 $\mu\text{g/mL}$ IS, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: μ Bondapak C18 Guard-Pak

Column: 100 \times 8 μ Bondapak C18 Radial-Pak

Mobile phase: MeOH:10 mM pH 2.69 KH_2PO_4 40:60

Flow rate: 2.8

Injection volume: 15

Detector: UV 229

CHROMATOGRAM

Retention time: 4.76

Internal standard: methyl-4-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]butyrate hydrochloride (8.36)

KEY WORDS

stability-indicating; 5% dextrose; injections

REFERENCE

Wiest,D.B.; Garner,S.S.; Childress,L.M. Stability of esmolol hydrochloride in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1995**, 52, 716–718.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 3.9 μm Nova-Pak C18

Mobile phase: MeOH:buffer 30:70 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 2.86 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: k' 4.42

OTHER SUBSTANCES

Also analyzed: acebutolol, bunitrolol, carazolol, celiprolol, mepindolol, metoprolol, timolol

REFERENCE

Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β -adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, 32, 14[94]20.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 12 μ m 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: k' 4.43

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleppamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1 -acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, 9, 211–215.

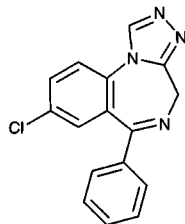
Estazolam

Molecular formula: C₁₆H₁₁ClN₄

Molecular weight: 294.74

CAS Registry No.: 29975-16-4

Merck Index: 3744



SAMPLE

Matrix: blood

Sample preparation: 500 μ L Serum + 20 μ L 20 μ g/mL IS + 200 μ L 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μ L mobile phase, inject a 20 μ L aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 \times 4.6 2 μ m TSK gel Super-ODS (A) or 100 \times 4.6 5 μ m Hypersil ODS-C18 (B)

Mobile phase: MeCN:5 mM pH 6 NaH₂PO₄ 45:55

Flow rate: 0.65

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 10.7 (A), 41.3 (B)

Internal standard: diazepam (29.8 (A), 77.5 (B))

Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam

Simultaneous: alprazolam

Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2- μ m porous microspherical silica gel, *J.Chromatogr.B*, **1996**, 682, 173–178.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 2 mL water + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μ L mobile phase, vortex for 30 s, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 50 \times 4.6 Shim-pack FLC-C8 (Shimadzu)

Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na₂HPO₄ adjusted to pH 6.0 with phosphoric acid.)

Flow rate: 0.6

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 4

Internal standard: estazolam

OTHER SUBSTANCES

Extracted: diazepam, nordiazepam, clorazepate, temazepam, oxazepam

Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam

Noninterfering: haloperidol, trihexyphenidyl

Interfering: triazolam

KEY WORDS

serum; estazolam is IS

REFERENCE

Tada,K.; Moroji,T.; Sekiguchi,R.; Motomura,H.; Noguchi,T. Liquid-chromatographic assay of diazepam and its major metabolites in serum, and application to pharmacokinetic study of high doses of diazepam in schizophrenics, *Clin.Chem.*, **1985**, 31, 1712–1715.

SAMPLE

Matrix: blood

Sample preparation: 500 μ L Plasma + 20 μ L 2.5 μ g/mL norprazepam in MeOH + 50 μ L buffer + 6 mL diethyl ether:dichloromethane 2:1, agitate, centrifuge. Remove the organic phase and evaporate to dryness under vacuum at 45°, dissolve the residue in 50 μ L MeOH, inject a 20 μ L aliquot. (Prepare buffer as follows. Solution A was 6.18 g boric acid + 7.46 g KCl in 100 mL water. Solution B was 10.6 g sodium carbonate in 100 mL water. Mix 63 mL solution A and 37 mL solution B and adjust pH to 9.5.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Nova Pak C18

Mobile phase: MeCN:MeOH:buffer 23:13:64 (Buffer was 94 mL 200 mM NaH₂PO₄ + 6 mL 200 mM Na₂HPO₄, adjusted to pH 5.0 with 100 mM HCl.)

Flow rate: 1.3
Injection volume: 20
Detector: UV 242

CHROMATOGRAM

Retention time: 10.2
Internal standard: norprazepam (18.6)
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: alprazolam, bromazepam, chlordiazepoxide, clobazam, diazepam, flumazenil, flunitrazepam, loflazepate, nitrazepam, norflunitrazepam, tofizopam, triazolam

Noninterfering: acepromazine, aceprometazine, amylobarbitol, aprobarbital, barbital, brallobarbitol, butalbital, caffeine, carbamazepine, chlorpromazine, cyclobarbitol, ethosuximide, heptabarbitol, hexobarbitol, loprazolam, medazepam, midazolam, pentobarbital, phenobarbital, phenytoin, prazepam, secobarbital, theophylline, thiopental, vinylbarbital

Interfering: oxazepam, clonazepam, lorazepam

KEY WORDS

plasma

REFERENCE

Boukhabza, A.; Lugnier, A. A.; Kintz, P.; Mangin, P. Simultaneous HPLC analysis of the hypnotic benzodiazepines nitrazepam, estazolam, flunitrazepam, and triazolam in plasma, *J. Anal. Toxicol.*, **1991**, *15*, 319–322.

SAMPLE

Matrix: blood

Sample preparation: Inject 100–200 μ L plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES

Column: A 45 \times 4 12 μ m TSK-gel G 3 PW (Tosohass); B 75 \times 4.6 Ultrasphere ODS C18 3 μ m
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.

Flow rate: 1

Injection volume: 100–200

Detector: UV 230

CHROMATOGRAM

Retention time: 21.5

OTHER SUBSTANCES

Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clonazepam, desmethyloclobazam, desmethyldiazepam, diazepam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam

Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid

Interfering: oxazepam

KEY WORDS

plasma; column-switching

REFERENCE

Lacroix, C.; Wojciechowski, F.; Danger, P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J. Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 223

CHROMATOGRAM

Retention time: 3.94

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoyllecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melfalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrridine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenopropfen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

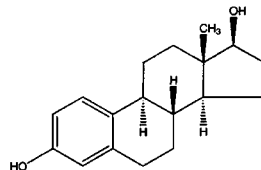
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE**Matrix:** blood, urine**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)**HPLC VARIABLES****Guard column:** 20 mm long Symmetry C18**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.**Column temperature:** 30**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)**Injection volume:** 10-30**Detector:** UV 221.6**CHROMATOGRAM****Retention time:** 16.495**KEY WORDS**

whole blood

REFERENCEGaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

Estradiol

Molecular formula: C₁₈H₂₄O₂**Molecular weight:** 272.39**CAS Registry No.:** 50-28-2, 113-38-2 (dipropionate), 979-32-8 (valerate), 57-91-0 (α- estradiol), 50-50-0 (benzoate), 313-06-4 (cypionate), 4956-37-0 (enantate), 3571-53-7 (undecylenate)**Merck Index:** 3746**Lednicer No.:** 1 162; 2 136**SAMPLE****Matrix:** blood**Sample preparation:** 1 mL Serum + 1 mL 1 mM tetrapentylammonium bromide in 1 M NaOH, mix, add 5 mL 1 mM 1-pyrenesulfonyl chloride (Molecular Probes, Eugene OR) in dichloromethane, vortex for 10 min, centrifuge at 1800 rpm for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in mobile phase, inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 250 × 4.6 5 µm Ultramex C8**Mobile phase:** MeCN:water 75:25**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 348, F ex 350 em 385, F ex 325 (Ar laser)

CHROMATOGRAM**Retention time:** 8

OTHER SUBSTANCES**Simultaneous:** equilin, estrone

KEY WORDS

derivatization; serum

REFERENCE

DeSilva, K.H.; Vest, F.B.; Karnes, H.T. Pyrene sulphonyl chloride as a reagent for quantitation of oestrogens in human serum using HPLC with conventional and laser-induced fluorescence detection, *Bio-med. Chromatogr.*, **1996**, 10, 318–324.

SAMPLE**Matrix:** blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES**Guard column:** 20 mm long Symmetry C18**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10–30**Detector:** UV 200.5

CHROMATOGRAM**Retention time:** 18.202

KEY WORDS

whole blood

REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149–163.

SAMPLE**Matrix:** solutions

Sample preparation: Add 25 mL receptor fluid to a 100 mg LiChrolut RP 18 SPE cartridge using vacuum. Elute with 4 mL MeCN, evaporate the eluate, reconstitute the residue in 1 mL MeCN. Inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Lichrospher 100 RP C18**Mobile phase:** MeCN:water 60:40**Column temperature:** 30**Flow rate:** 1.0**Detector:** F ex 225 no emission filter

KEY WORDS

SPE

REFERENCE

Rohr,U.D.; Altenburger,R.; Kissel,T. Pharmacokinetics of the transdermal reservoir membrane system delivering β -estradiol: In vitro/in vivo-correlation, *Pharm.Res.*, **1998**, *15*, 877-882.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 200 \times 4.6 5 μ m Hypersil ODS**Mobile phase:** MeCN:water 60:40**Column temperature:** 37**Flow rate:** 1.5**Detector:** UV 226

CHROMATOGRAM**Retention time:** 2.34

OTHER SUBSTANCES**Simultaneous:** progesterone

REFERENCE

Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, *85*, 1191-1195.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 300 \times 3.9 μ Bondapak C18**Mobile phase:** MeCN:50 mM pH 6 potassium phosphate buffer 47:53**Flow rate:** 1.5**Detector:** UV 208

CHROMATOGRAM**Retention time:** 13-14**Internal standard:** estradiol-3-acetate

OTHER SUBSTANCES**Simultaneous:** mestranol, 17 α -ethinyl estradiol**Noninterfering:** ketoconazole, fluconazole, itraconazole, miconazole, α -naphthoflavone, quinine, sulfaphenazole, troleandomycin

KEY WORDS

estradiol-3-acetate is IS

REFERENCE

Schmider,J.; Greenblatt,D.J.; von Moltke,L.L.; Karsov,D.; Vena,R.; Friedman,H.L.; Shader,R.I. Biotransformation of mestranol to ethinyl estradiol in vitro: The role of cytochrome P-450 2C9 and metabolic inhibitors, *J.Clin.Pharmacol.*, **1997**, *37*, 193-200.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Ultrasphere**Mobile phase:** MeCN:EtOH:water 54:1:45

Flow rate: 1.5
Detector: UV 270

CHROMATOGRAM

Retention time: 2.4 (17 β -estradiol)

REFERENCE

Fridriksdottir,H.; Loftsson,T.; Gudmundsson,J.A.; Bjarnason,G.J.; Kjeld,M.; Thorsteinsson,T. Design and in vivo testing of 17 β -estradiol-HP β CD sublingual tablets, *Pharmazie*, **1996**, 51, 39–42.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μ L water to the residue, extract twice with 2 mL portions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelco silica

Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.

Flow rate: 2.0

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 10.25

Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: diethylstilbestrol, zeranol

Simultaneous: estrone, zeralenol, zeralenone, zeralanone

KEY WORDS

chicken; muscle; normal phase; SPE

REFERENCE

Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17 β -oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, 3, 263–272.

Estramustine

Molecular formula: C₂₃H₃₁Cl₂NO₃

Molecular weight: 440.41

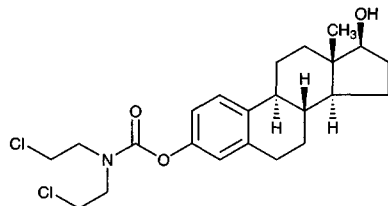
CAS Registry No.: 2998-57-4, 52205-73-9
(phosphate sodium)

Merck Index: 3749

Lednicer No.: 3 83

SAMPLE

Matrix: blood



Sample preparation: 1 mL Plasma + 100 μ L EtOH + 2 mL buffer, mix well, add 12 mL hexane, shake slowly for 15 min on a reciprocating shaker, centrifuge at 1200 g at 5° for 10 min. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L hexane:EtOH 92.5:7.5, inject a 20 μ L aliquot. (Buffer was 630 mL of a solution containing 61.8 g/L boric acid and 74.6 g/L KCl and 370 mL 106 g/L sodium carbonate solution, shake well, adjust pH to 9.0 with sodium carbonate solution (if necessary), store at 35-37°.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil PXS 5/25 silica gel

Mobile phase: Hexane:EtOH 92.5:7.5

Flow rate: 1.5

Injection volume: 20

Detector: F ex 195 em 250 (cut-off filter)

CHROMATOGRAM

Retention time: 7.2

Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: estrone, estradiol

KEY WORDS

plasma; normal phase; normal phase more sensitive than reverse phase; pharmacokinetics

REFERENCE

Brooks,M.A.; Dixon,R. Determination of estramustine and its 17-keto metabolite in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, 182, 387-394.

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 20-50 μ L aliquot. (Hydrolyze 500 μ L plasma by adding 500 μ L 200 mM pH 5 acetate buffer and 100 μ L beef liver β -glucuronidase (Sigma) or 10 μ L β -glucuronidase/sulfatase (Glusulase), heat at 37° overnight, add 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 20-50 μ L aliquot.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil 5/25 silica gel

Mobile phase: Hexane:EtOH 92.5:7.5

Flow rate: 1.5

Injection volume: 20-50

Detector: F ex 195 em 250 (cut-off filter)

CHROMATOGRAM

Retention time: 7.2

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: estromustine, estrone, estradiol, metabolites

KEY WORDS

plasma; rat; dog; human; pharmacokinetics; normal phase

REFERENCE

Dixon,R.; Brooks,M.; Gill,G. Estramustine phosphate: Plasma concentrations of its metabolites following oral administration to man, rat and dog, *Res.Commun.Chem.Pathol.Pharmacol.*, **1980**, 27, 17-29.

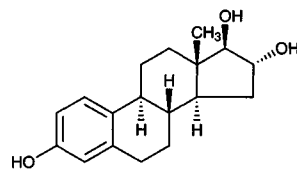
Estriol

Molecular formula: $C_{18}H_{24}O_3$

Molecular weight: 288.39

CAS Registry No.: 50-27-1, 113-22-4
(16,17-bis(sodium hemisuccinate))

Merck Index: 3750



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 \times 4.6 5 μ m Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.142

KEY WORDS

whole blood

REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

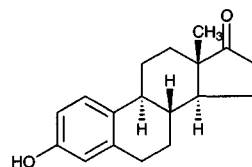
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitrityline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glycbenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetidine, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233-242.

Estrogens, conjugated



Molecular formula: $C_{18}H_{18}O_2$ (equilenin), $C_{18}H_{22}O_2$ (17α -dihydroequilin), $C_{18}H_{20}O_2$ (equilin), $C_{18}H_{22}O_2$ (estrone), $C_{18}H_{24}O_2$ (estradiol)

Molecular weight: 266.34 (equilenin), 272.39 (estradiol), 268.36 (equilin), 270.37 (17α -dihydroequilin), 270.39 (estrone)

CAS Registry No.: 474-86-2 (equilin), 50-28-2 (estradiol), 57-91-0 (α -estradiol), 517-09-9 (equilenin), 53-16-7 (estrone), 338-67-5 (estrone sodium sulfate), 481-97-0 (estrone hydrogen sulfate)

Merck Index: 3216 (17 α -dihydroequilin), 3675 (equilenin), 3676 (equilin), 3746 (estradiol), 3751 (estrone)

Lednicer No.: 1 156 (estrone); 1 162 (estradiol); 2 136 (estradiol)

SAMPLE

Matrix: blood

Sample preparation: 0.5-1 mL Plasma + 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 20-50 μ L aliquot. (Hydrolyze 500 μ L plasma by adding 500 μ L 200 mM pH 5 acetate buffer and 100 μ L beef liver β -glucuronidase (Sigma) or 10 μ L β -glucuronidase/sulfatase (Glusulase), heat at 37° overnight, add 1 mL 500 mM pH 7 phosphate buffer + 12 mL hexane:ethyl acetate 70:30, extract. Remove a 10 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μ L mobile phase, inject a 20-50 μ L aliquot.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Partisil 5/25 silica gel

Mobile phase: Hexane:EtOH 92.5:7.5

Flow rate: 1.5

Injection volume: 20-50

Detector: F ex 195 em 250 (cut-off filter)

CHROMATOGRAM

Retention time: 6.2 (estrone)

Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: estromustine, estradiol, estramustine, metabolites

KEY WORDS

plasma; rat; dog; human; pharmacokinetics; normal phase

REFERENCE

Dixon,R.; Brooks,M.; Gill,G. Estramustine phosphate: Plasma concentrations of its metabolites following oral administration to man, rat and dog, *Res.Commun.Chem.Pathol.Pharmacol.*, **1980**, 27, 17-29.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 5 mL water + 1 mL 2 μ g/mL equilenin in MeOH + 50 μ L 0.1 M NaOH to adjust pH to 10, vortex briefly after each addition, shake with 10 mL dichloromethane for 10 min, centrifuge at 2000 g for 10 min. Wash organic layer twice with 2 mL water, centrifuge 5 min, evaporate 8 mL of organic phase to dryness at 40° under a stream of nitrogen, reconstitute residue in 150 μ L mobile phase, inject 25 μ L aliquot

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeOH:buffer 65:35 (Buffer was 10 mL 200 mM acetic acid + 15 mL 200 mM sodium acetate made up to 1 L, pH 4.8.)

Flow rate: 1

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Retention time: 5.3 (equilenin)

Internal standard: equilenin

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: hydrocortisone, deoxycortisol, triamcinolone, prednisone, dexamethasone, betamethasone

KEY WORDS

plasma; equilenin is IS

REFERENCE

Bouquet,S.; Brisson,A.M.; Gombert,J. Dosage du cortisol et du 11-désoxycortisol plasmatiques par chromatographie liquide haute performance [Cortisol and 11-desoxycortisol determination in blood by high performance liquid chromatography], *Ann.Biol.Clin.(Paris)*, **1981**, 39, 189–191.

SAMPLE

Matrix: blood

Sample preparation: 100 μ L Serum + 500 μ L water + 100 μ L 10 μ g/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L MeOH:water 40:60, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m NS-Gel C18

Mobile phase: Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min

Column temperature: 50

Flow rate: 1

Injection volume: 50

Detector: UV 210, UV 240

CHROMATOGRAM

Retention time: 28.38 (estrone)

Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES

Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, hydrocortisone, 17-hydroxyprogesterone, pregnenolone, progesterone

KEY WORDS

serum

REFERENCE

Ueshiba,H.; Segawa,M.; Hayashi,T.; Miyachi,Y.; Irie,M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method, *Clin.Chem.*, **1991**, 37, 1329–1333.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 3 μ m Nucleosil C18

Mobile phase: MeOH:dichloromethane:2-propanol:water 45:5:7.5:42.5

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 16.80 (17 α -dihydroequilenin), 19.30 (17 α -dihydroequilin), 22.52 (17 α -estradiol), 25.77 (equilenin), 28.62 (equilin), 32.19 (estrone)

REFERENCE

Novakovic,J.; Pacáková,V.; Sevcik,J.; Cserhádi,T. Quantitative structure-chromatographic retention relationship study of six underivatized equine estrogens, *J.Chromatogr.B*, **1996**, 681, 115–123.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 1.43 (estrone)

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 µm Nucleosil C18

Mobile phase: MeCN:THF:water 12.9:22.4:64.7

Flow rate: 1

Detector: UV 240

CHROMATOGRAM

Retention time: 11.5 (estrone)

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, mestranol, norethindrone, norethindrone acetate, norgestrel

REFERENCE

Gazdag,M.; Szepesi,G.; Szelecski,E. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. I. Optimization for selectivity in reversed-phase chromatography, *J.Chromatogr.*, **1988**, 454, 83–94.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm LiChrosorb Si 60

Mobile phase: Hexane:dioxane:isopropanol 95:3:2

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 11 (estrone)

OTHER SUBSTANCES

Simultaneous: ethinyl estradiol, mestranol, norethindrone, norethindrone acetate, norgestrel

KEY WORDS

normal phase

REFERENCE

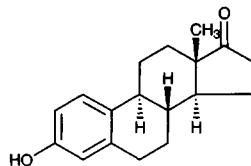
Gazdag,M.; Szepesi,G.; Fábián-Varga,K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. II. Optimization for selectivity in normal-phase systems, *J.Chromatogr.*, **1988**, 454, 95–107.

SAMPLE**Matrix:** solutions**Sample preparation:** Inject an aliquot of a solution in MeOH.**HPLC VARIABLES****Column:** Radial-PAK μ Bondapak C18**Mobile phase:** MeCN:water 50:50**Flow rate:** 2**Injection volume:** 100**Detector:** UV 254 or 214**CHROMATOGRAM****Retention time:** 2.5 (estriol), 5.5 (testosterone), 5.6 (17 β -estradiol), 6.9 (estrone), 16.3 (progesterone)**KEY WORDS**

testosterone and estradiol interfere

REFERENCEErkoc,F.U.; Özsar,S.; Güven,B.; Kalkandelen,G.; Ugrar,E. High-performance liquid chromatographic analysis of steroid hormones, *J.Chromatogr.Sci.*, **1989**, 27, 86–90.**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare an aqueous solution, inject a 20 μ L aliquot.**HPLC VARIABLES****Column:** 150 \times 4.6 3.5 μ m Zorbax SB C18**Mobile phase:** MeCN:MeOH:buffer 15:45:40 (Buffer was 10 mM KH₂PO₄ and 50 mM tetrabutylammonium chloride, pH adjusted to 3.0 with 1 M HCl.)**Flow rate:** 0.9**Injection volume:** 20**Detector:** UV 220**CHROMATOGRAM****Retention time:** 11.2 (estrone), 8.4 (estrone-3-phosphate)**OTHER SUBSTANCES****Simultaneous:** estriol, 17 β -estradiol, 17 β -estradiol-3-phosphate**REFERENCE**Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of 17 β -estradiol-3-phosphate in an ophthalmic solution, *Chromatographia*, **1995**, 40, 204–206.

Estrone

Molecular formula: C₁₈H₂₂O₂**Molecular weight:** 270.37**CAS Registry No.:** 53-16-7, 338-67-5 (sodium sulfate), 481-97-0 (hydrogen sulfate)**Merck Index:** 3751**Lednicer No.:** 1 156**SAMPLE****Matrix:** blood